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Fine-Tuning Motor Neuron Properties: Signaling from the Periphery

Minireview

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Our understanding of motor neuron differentiation is rapidly evolving. New studies demonstrate that cells in the periphery of the embryo provide feedback signals for spinal cord motor neurons that are instrumental in the timing and regulation of their development. Two papers in this issue of *Neuron* (Haase et al., 2002; Livet et al., 2002) identify a motor neuron survival factor, GDNF, and the ETS transcription factor, PEA3, as key components of a signal transduction pathway whose goals are 2-fold: to cluster motor pool-specific cell bodies and to promote axon arborization.

Introduction

During the development of the spinal cord, individual subtypes of motor neurons extend axons into the periphery to form synaptic connections with specific muscles and thereby control locomotion. The activity of motor neurons is regulated by a number of inputs, including rostrally located neurons whose axons descend into the spinal cord, local spinal interneurons, and proprioceptive sensory neurons situated within the dorsal root ganglia (DRG). Most motor neuron subtypes arise from a common source of progenitor cells within the ventral neural tube and therefore start out as intermingled cell populations. However, as development progresses, these neurons segregate into specific columns and sort into pools in a very stereotyped manner (Jessell, 2000).

Motor neurons located within the same motor pools share many common properties: they have similar LIM, ETS, and cadherin gene expression profiles (Arber et al., 2000; Lin et al., 1998; Price et al., 2002; Tsuchida et al., 1994), they innervate the same muscle, they receive synaptic inputs from sensory afferents innervating the homonymous muscle, they are electrically coupled through gap junctions (Chang and Balice-Gordon, 2000), their axons tend to arborize to a similar degree (Haase et al., 2002; Livet et al., 2002), and as the name implies, they pool together in a similar position within the spinal cord. Interestingly, recent studies have found that these latter two attributes of motor pools seem to be modified by interactions between motor axons and the periphery.

The establishment of connectivity between motor neurons and muscles can be broken down into sequential modular steps (Figure 1A) (Livet et al., 2002). Motor axons exit the spinal cord through the ventral roots. Once in the periphery, axons select among major pathways into the limb or toward axial muscles, for example. Next, specific muscle targets are chosen, then axons invade and arborize within the muscle in a stereotyped manner. After contacting their targets, motor neurons become trophically dependent and programmed cell death ensues, eliminating about half the cells. Finally, a rearrangement of synapses occurs in which polysynaptically innervated muscle fibers become monosynaptically innervated. Because these events occur in a strict developmental progression, it is logical to expect that the genetic programs controlling each of these events will be regulated in a precise manner in order to maintain the proper developmental sequence.

Early axonal projections out of the spinal cord are programmed by combinations of LIM homeodomain transcription factors (Sharma et al., 1998). LIM factors also control the selection of major nerve pathways into the limb and axial musculature (Shirasaki and Pfaff, 2002). What has remained less clear are the mechanisms that establish motor pools and the regulation of the later steps in motor neuron differentiation. In this review, we describe new findings that provide insight into the later process of axonal arborization, a seemingly generic step in motor neuron differentiation that is found to be regulated by glial cell line-derived neurotrophic factor (GDNF) and pool-specific ETS transcription factors.

ETS Regulation of Sensory Afferent and Motor Pool Differentiation

PEA3 and ER81 have a complex temporal and spatial expression pattern in DRG sensory neurons involved in the stretch reflex circuitry (Arber et al., 2000; Lin et al., 1998). Arber and colleagues demonstrated that the ETS transcription factor, ER81, is necessary for proprioceptive sensory neurons to extend and branch into the ventral spinal cord in order to synapse with motor neurons (Arber et al., 2000) (Figure 1B). Although terminal branching is affected in *Er81* mutant mice, the initial entry of sensory axons into the dorsal spinal cord remains unperturbed (Arber et al., 2000). Thus, ER81 is nonessential for axon growth, but rather controls the terminal branching pattern of sensory neurons.

Haase et al. (2002) and Livet et al. (2002) report that PEA3 and GDNF are necessary for the proper axonal arborization of motor neurons—specifically the motor neurons that innervate the cutaneous maximus (CM) and latissimus dorsi (LD) muscles. Thus, without PEA3 or GDNF, the CM and LD are incompletely innervated (Figure 1B). As in the *Er81* mutant sensory neurons, the initial axonal growth and trajectories are unperturbed in *GDNF* and *Pea3* mutant motor neurons, but arborization is severely reduced or absent.

In conjunction with the LIM factors, PEA3 and ER81 mark individual motor pools and specific populations of proprioceptive sensory neurons. Interestingly, studies of chick embryos have found that monosynaptically connected motor and sensory neurons are matched in their profiles of ETS gene expression (Lin et al., 1998). Therefore, it has been hypothesized that these genes mediate the proper connections between these two cell types, perhaps by regulating the expression of molecules that interact homophilically. Moreover, the similarities in the axon branching defects in *Er81* and *Pea3* mutant mice raise the possibility that these transcription factors regulate overlapping sets of genes. Defining the target genes of ER81 and PEA3, and examining sensory branches in

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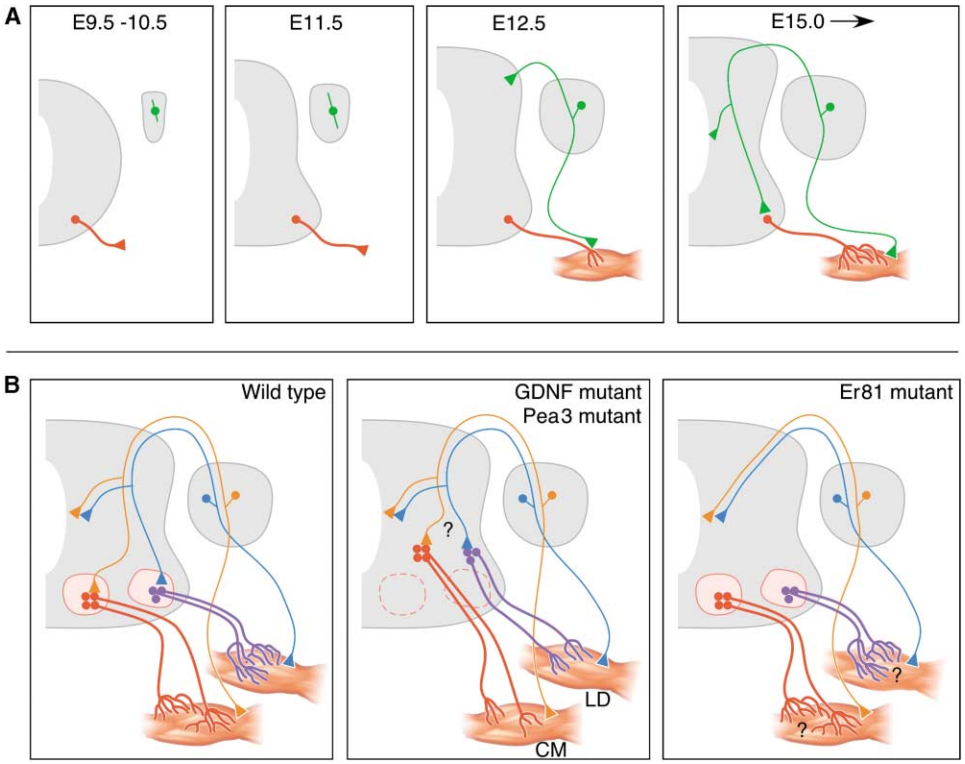

Figure 1. The Role of GDNF and ETS Transcription Factors in the Sequential Development of Monosynaptic Stretch Reflex Circuits

(A) Sequential steps in the development of motor neurons (red) and sensory afferents (green) (Arber et al., 2000; Haase et al., 2002; Livet et al., 2002). *Pea3* expression begins at E10.5 in motor neurons and E12.5 in sensory neurons. The onset of *Er81* is E12.0 in motor neurons and E13.0 in sensory neurons. After E12.5, arborization of muscle targets appears. At E14.0, proprioceptive afferents project into the dorsal spinal cord. At E15.0, afferents project to the intermediate (Clarke's column) spinal cord area and by E15.5, sensory afferents extend ventrally, proximal to motor dendrites and cell bodies. Direct functional connections between sensory and motor neurons occur from E18–P2.

(B) Summary of the phenotypes observed in *GDNF* (Haase et al., 2002), *Pea3* (Livet et al., 2002), and *Er81* (Arber et al., 2000) mutant mice. Both PEA3 and ER81 are present in motor and sensory neurons. GDNF is expressed in the limb bud. GDNF receptors are expressed in motor neurons. In *Pea3* and *GDNF* mutants, arborization within CM and LD muscles is abnormal and motor neuron cell bodies are mispositioned. In *Er81* mutants, ventral projections of sensory afferents within the spinal cord are absent. The central sensory projections of *GDNF* and *Pea3* mutants as well as the arborization of motor neurons in different muscles of *Er81* mutants requires detailed examination.

Pea3 mutants and motor branches in *Er81* mutants, will elucidate the apparent similarities and differences in the function of related ETS transcription factors.

Regulation of ETS Expression

The periphery is an important regulator of ETS genes in LMC motor neurons and DRG sensory neurons. This was found by ablating the limb bud and noting the loss of ETS expression (Lin et al., 1998). Haase et al. have now identified the peripheral signal for inducing PEA3 expression in motor neurons as GDNF—glial cell line-derived neurotrophic factor. GDNF is expressed in peripheral mesenchymal and muscle cells encountered by the growing axons of the LMC motor neurons. As expected, the expression of GDNF slightly precedes that of PEA3, and mutants of *GDNF* are unable to trigger normal levels of PEA3 expression in motor neurons (Haase et al., 2002).

Previous studies demonstrated that GDNF is a potent motor neuron survival factor (Airaksinen and Saarma, 2002). This trophic dependency is surprisingly complex (Garces et al., 2000). Motor neurons appear to acquire trophic dependency sometime after they are generated, around the time their axons approach muscles. An additional complexity is that the trophic signal does not appear to be a universal one for all motor neurons, rather it appears that a composite of many different factors, including GDNF, maintains motor neuron survival (Kaplan and Miller, 2000). There is a family of GDNF ligands, distantly related to the TGF-β superfamily, composed of GDNF, neurturin (NRTN), persephin (PSPN), and artemin (ARTN) (Airaksinen and Saarma, 2002). Gfrα1-4 are GPI-anchored receptors that selectively interact with the four ligands (Figure 2A). The Ret tyrosine kinase represents a coreceptor for all of the Gfrαs. Signaling is thought to occur when GDNF homodimers bind to Gfrα1 receptors that in turn interact with the Ret coreceptors which transduce the signal via phosphorylation of intracellular proteins (Figure 2B).

Early in development at E10, GDNF is expressed at the base of the limb where many subtypes of brachial motor axons converge before growing toward more distal targets (Haase et al., 2002). A day or two later, the CM and LD muscles begin to form and also express GDNF. These observations raise the question of why GDNF appears to be selective for CM and LD innervating motor neurons when the receptor components are expressed by other motor neuron subclasses that encounter GDNF at the base of the limb. Haase et al. examined explants of the neural tube containing different motor neuron subtypes and found that GDNF induces PEA3 expression only in motor pools fated to express PEA3 in vivo. Therefore, the expression of GDNF receptor

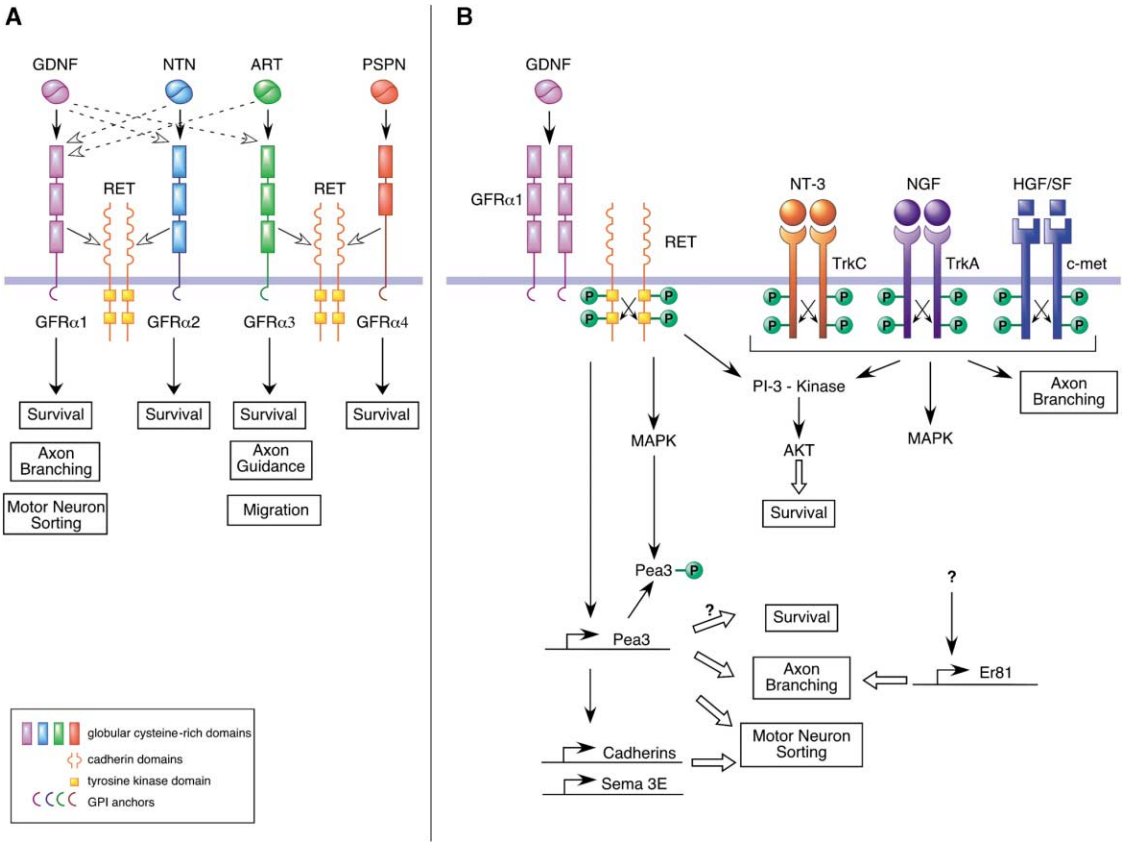

Figure 2. GDNF Signaling and the Induction of *Ets* Genes

(A) GDNF family members, their cognate receptors, and their potential for cross-interactions (Airaksinen and Saarma, 2002; Garces et al., 2000; Oppenheim et al., 2000).

(B) Hypothetical pathway for GDNF (Airaksinen and Saarma, 2002; Soler et al., 1999), NT-3, NGF, and HGF/SF signaling (Kaplan and Miller, 2000). ETS factors are phosphorylated by MAPK and the role of phosphorylation is yet to be determined in motor neuron differentiation. The RET, TrkC, TrkA, and c-met receptors also signal through the PI-3 kinase pathway resulting in cell survival.

complexes in motor neurons does not guarantee induction of PEA3. The apparent selectivity for GDNF signaling in motor neuron subtypes may be due to a number of reasons such as timing, affinity and activity of receptors in motor neuron subsets, and/or repression of GDNF activity in specific cells (Haase et al., 2002).

Although PEA3 expression is dramatically reduced in *GDNF* mutants, approximately 10% of the motor neurons retain PEA3 in the absence of GDNF, and DRG neurons continue to express the ETS factor in *GDNF* mutants (Haase et al., 2002). This raises the possibility that the other GDNF family members may also contribute to the induction of PEA3 expression in sensory and motor neurons. While GDNF binds the GFRα1 receptor with highest affinity, it can crosstalk with GFRα2 and GFRα3 receptors (Airaksinen and Saarma, 2002) (Figure 2A). Similarly, NRTN and ARTN can also crosstalk with the GFRα1 receptor. In *GDNF* mutant mice, Gfrα2 transcripts are upregulated (Oppenheim et al., 2000), suggesting that Gfrα2 and other ligands might be able to partially compensate for the loss of GDNF. Further analysis of the potential for redundancy or compensatory mechanisms with GDNF-related signaling components will show the full range of functions that this signaling pathway has on motor neuron development.

What about the relationship between GDNF, PEA3, and cell survival? In *GDNF* and *Gfrα1* mutant mice, motor neuron numbers are reduced by 25% compared to wild-type embryos following the natural cell death period. At present, it is unclear whether PEA3 mediates the survival effect of GDNF (Figure 2B). Although the loss of PEA3 function in motor neurons does not result in premature cell death, neither does the loss of GDNF. To understand the relationship between GDNF and PEA3 in survival, it will be important to examine and compare motor neuron numbers after the natural cell death period in *Pea3* and *GDNF* mutants. In the future, a more detailed understanding of the GDNF signaling pathway will help reveal how GDNF acts within subsets of motor neurons and how it controls both axon arborization and cell survival, and help define how these two processes are related (Figure 2B).

GDNF: Branch Promotion and Putative Downstream Effectors

Mutants in *GDNF* and *Pea3* demonstrate compromised axon branching patterns. Conversely, the ectopic expression of GDNF in muscles of transgenic mice using the myogenin promoter results in increased axonal branching in the sternomastoideus and spinotrapezius muscles (Keller-Peck et al., 2001; Nguyen et al., 1998). Revisiting these experiments and determining if PEA3 is induced in the neurons that innervate these muscles

could prove to be interesting. The branch promotion property of GDNF is shared with other neurotrophic factors such as hepatocyte growth factor/scatter factor (HGF/SF) (Ebens et al., 1996), nerve growth factor (NGF) (Kennedy and Tessier-Lavigne, 1995), and neurotrophin-3 (NT-3) (Kennedy and Tessier-Lavigne, 1995) (Figure 2B). Determining if there is a relationship between these other neurotrophic signals and ETS regulation might be informative.

Surprisingly, the loss of GDNF and PEA3 function reveals branching defects within specific areas of the CM and LD muscles. This is unexpected because GDNF appears to be expressed evenly throughout CM and LD muscles, not in a graded fashion (Haase et al., 2002; Livet et al., 2002). Likewise, PEA3 appears to be expressed without bias in branches of nerves innervating different areas of the LD and CM muscles. While it is possible that the GDNF/PEA3 pathway is part of a simple signal for final extension and arborization, the selectivity of the mutant phenotype suggests that it might also be part of a sophisticated topographical cue delineating muscle regions. It is already well established that motor neuron innervation of muscles is highly ordered, and Ephrins have been shown to be important in the topographic mapping of motor axon synapses onto muscle fibers (Feng et al., 2000). Perhaps this mapping process is dependent upon an interplay between positive-acting signals such as GDNFs and negative signals such as Ephrins.

What would be the logic of using specific transcription factors restricted to subsets of motor neurons for something as generic as branching? The ETS transcription factors may provide specificity within a more general program of neuronal differentiation. It is thought that all neurons possess some generic arborization program, but perhaps with larger muscle surfaces it is necessary to superimpose additional programs regulated by the ETS factors to ensure that larger targets are properly innervated (Livet et al., 2002). This in turn raises the question of whether other apparently general aspects of neuronal differentiation are likewise regulated by specific transcription factors. Feedback signaling represents an elegant mechanism for achieving the fine-tuning of a neuron's properties, in this example, matching arborization to the size of the postsynaptic field.

What are the downstream effectors allowing axons to arborize at synapses and cluster together in pools? The connection between cadherins and ETS genes was made in chick experiments by Price et al. where they found that Type II cadherins influence motor pool segregation. Their studies showed that specific cadherins delineate motor pools and that altering these patterns leads to defects in motor neuron sorting. It is likely, therefore, that the cadherins interact in a homophilic manner to facilitate the normal positioning of motor neuron cell bodies. The relationship between ETS transcription factors and cadherin regulation has been made in several ways. Ectopic ER81 expression disregulates cadherin expression in motor pools (Price et al., 2002), whereas cadherin-8 expression is lost in *Pea3* mutants (Livet et al., 2002). In a manner that is reminiscent of the cadherin phenotypes in chick embryos, motor pools become mispositioned and disorganized in both *GDNF* and *Pea3* mutants. Direct analysis of the cadherin promoter has led to the identification of ETS binding sites (Gory et al., 1998). Together, these results provide an interesting link between the peripheral signal, GDNF, and neuronal positioning within the spinal cord as mediated by ETS transcription factors and cadherins.

These two recent studies have shown that motor neuron differentiation is more complex than previously appreciated. It is well established that a variety of spinal cord signals such as sonic hedgehog and retinoic acid lead to the generation of distinct motor neuron subtypes (Jessell, 2000), but it is now evident that further fine-tuning allows for very specific matching between motor neurons and specific muscles. Interestingly, the fine-tuning of motor pool segregation and axonal branching is mediated by retrograde signaling from the periphery back into the nucleus.

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